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Lynn Bergmeyer, et al
U.S. Serial No. US 062,021
Filed 14 May 1993
FOR: DIAGNOSTIC COMPOSITIONS,ELEMENTS,METHODS AND TEST KITS FOR AMPLIFICATION AND DETECTION OF HUMAN CMV DNA USING PRIMERS HAVING MATCHED MELTING TEMPERATU
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of

Lynn Bergmeyer
Thomas J. Cummins
John B. Findlay and
JoAnne Kerschner

DIAGNOSTIC COMPOSITIONS, ELEMENTS, METHODS AND TEST KITS FOR AMPLIFICATION AND DETECTION OF HUMAN CMV DNA USING PRIMERS HAVING MATCHED MELTING TEMPERATURES

Serial No. 08/062,021

Filed May 14, 1993

Examiner: L. Arthur

Group Art Unit: 1807

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Date: Sept. 13, 1994

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231
Sir:

APPELLANTS' BRIEF

TABLE OF CONTENTS

I. STATUS OF CLAIMS

This is an appeal from the Office Action mailed March 21, 1994 finally rejecting Claims 1-13, 15-20, 22-27 and 30-38 of the present application under 35 U.S.C. 103 and 112(2).

A Notice of Appeal was filed with a Certificate of Mailing on July 21, 1994.

II. STATUS OF AMENDMENTS

An amendment was filed under Rule 116 on June 20, 1994, cancelling Claims 9, 15, 32 and 34, amending Claim 38, and adding new Claims 39-42 to specific embodiments of the invention.

In the Advisory Action mailed July 19, 1994, the Examiner indicated that the Rule 116 amendment would not be entered because new Claim 39 allegedly raised a new issue for consideration.

Appellants responded with a second Rule 116 amendment, mailed July 28, 1994, making the same changes as before, but additionally correcting the defect in new Claim 39.

In her second Advisory Action, mailed August 12, 1994, the Examiner indicated that the second Rule 116 amendment was entered, overcoming objections to the specification and drawings, and overcoming the rejection of Claim 38 under Section 112(2). Claim 38 was therefore allowed, but Claims 1-8, 10-13, 16-20, 22-27, 30, 31, 33, and 35-37 and 39-42 remained rejected under Sections 103 and 112(2).

III. SUMMARY OF THE INVENTION

A. Background

Cytomegalovirus is a double stranded DNA virus, approximately 240,000 nucleotides in length, and belonging to the Herpes virus group. Human cytomegalovirus (hCMV) has become increasingly of interest in a wide variety of disease states. In the

United States, hCMV infects up to 1% of all newborns and results in significant mortality in congenitally infected infants. Infections from hCMV also have a major impact on immunocompromised patients, such as those afflicted with acquired immunodeficiency syndrome (AIDS) and recipients of transplanted organs. People infected with the disease often suffer impairment of some of their vital organs, including the salivary glands, eyes, brain, kidneys and liver. In children, the hCMV infection represents the leading infectious cause of mental retardation and nonhereditary sensineural deafness. Furthermore, hCMV is associated with a wide variety of classical syndromes including mononucleosis and interstitial pneumonia. Studies suggest that hCMV is latent in white blood cells, and that it has a potential to cause certain cancers.

Since hCMV is relatively common in people, a considerable effort has been made to isolate it and to diagnose its disease states. Efforts to produce vaccines or to treat the disease continue unabated.

Diagnosis of the presence of hCMV DNA by culture requires up to three weeks and is technically difficult and expensive. Immunological assays are not presently considered reliable. Earlier diagnosis could be important for treating immunosuppressed patients and for screening blood supplies. HCMV has a large linear double stranded DNA genome composed of two unique components as well as terminal repeat sequences.

Technology to detect minute quantities of nucleic acids (including hCMV DNA) has advanced rapidly over the last two decades including the development of highly sophisticated hybridization assays using probes in amplification techniques such as polymerase chain reaction (PCR). Researchers have readily recognized the value of such technology to detect diseases and genetic features in human or animal test specimens. The use of probes and primers in such technology is based upon the concept of complementarity, that is, the bonding of two strands of a nucleic acid by hydrogen bonds between

complementary nucleotides (also known as nucleotide pairs).

PCR is a significant advance in the art which allows detection of very small concentrations of a targeted nucleic acid. The details of PCR are described in a rapidly expanding volume of literature in this field. Without going into extensive detail, PCR involves hybridizing primers to the strands of a targeted nucleic acid (considered "templates") in the presence of a polymerization agent (such as a DNA polymerase) and deoxyribonucleoside triphosphates under the appropriate conditions. The result is the formation of primer extension products along the templates, the products having added thereto nucleotides which are complementary to the templates.

Once the primer extension products are denatured, one copy of each template has been prepared, and the cycle of priming, extending and denaturation can be carried out as many times as desired to provide an exponential increase in the amount of nucleic acid which has the same sequence as the target nucleic acid. In effect, the target nucleic acid is duplicated (or "amplified") many times so that it is more easily detected. Despite the broad and rapid use of PCR in a variety of biological and diagnostic fields, there are still practical limitations which must be overcome to achieve the optimum success of the technology. PCR also uses expensive reagents in a not always efficient manner.

It is well known that PCR is susceptible to a "carry-over" problem whereby amplified nucleic acids from one reaction may be inadvertently carried over into subsequent reactions using "fresh" PCR reaction mixtures, and thereby causing "false" positives when testing later specimens. This problem is one of the major reasons PCR has not yet been commercialized on a large scale. It is so serious, that a carryover into a laboratory or clinic could render that facility useless for further PCR assays.

One approach to this problem is to completely contain the reagents for each PCR procedure so no reagents or by-products can be carried over. Specially designed test packs or test devices have been constructed to contain PCR procedures for this reason. Such test packs are described in various publications, and are preferably, but not necessarily, used in combination with automatic PCR processing equipment. This equipment is characterized by its capability to simultaneously process several test specimens in separate test devices.

However, it would be highly desirable to detect a multiplicity of target nucleic acids (or a multiplicity of nucleic acid sequences in the same nucleic acid) in a single test device using a single specimen. This is referred to herein as "multiplexing". The multiple target nucleic acids could be from the same or different infectious agents. For example, one could target two different sequences of hCMV DNA in order to detect lower quantities of the infectious agent at an earlier stage of disease. In other situations, an assay may detect a single target nucleic acid, but also detect a "positive control" target in order to verify assay reliability.

For each specific target nucleic acid to be amplified and detected, a specific set of primers and a capture probe (a total of three oligonucleotides) are needed. Thus, the three oligonucleotides are complementary and specific to that targeted nucleic acid sequence. For example, in multiplexing, if three nucleic acid sequences are to be amplified and detected, typically three sets of primers and probes are needed, one set specific for each sequence. Normally, detection of the multiple sequences requires a multiplicity of test devices, and perhaps different sets of PCR conditions (that is, temperature and time conditions).

It would be desirable, however, to amplify and detect a plurality of target nucleic acids simultaneously in the same test device and using "universal" processing equipment and conditions. This cannot be done by merely selecting any set of primers and probes specific for each

target nucleic acid from conventional sources. Where processing equipment is used to process several test devices simultaneously, or a single test device is designed for multiplexing, the equipment must be adapted to provide optimum heating and cooling times and temperatures for each set of primers and probes, since they will all likely have individual optimum denaturaton temperatures. To adapt the equipment to randomly selected primers and probes in multiplexing would be a very expensive and cumbersome solution to the problem. Yet there is a great need for efficient, relatively inexpensive and rapid multiplexing to detect multiple nucleic acid sequences of hCMV DNA, or one or more nucleic acid sequences of hCMV DNA and one or more nucleic acid sequences of other target nucleic acids.

B. Statement of the Invention

The present invention provides an effective and efficient means for multiplexing, or amplifying and detecting a multiplicity of target nucleic acid sequences using the same test device, if desired, and the same processing equipment. It is particularly useful for the detection of one or more nucleic acid sequences of hCMV DNA alone, and one or more nucleic acid sequences of one or more additional target nucleic acids. Any number of nucleic acid sequences can be amplified and determined simultaneously using the appropriate primer sets and probes in combination.

Thus, the problem noted above about the need for a means to "multiplex" using a single PCR apparatus and test device, is solved with the present invention.

The solution is achieved by using a set of "matched" primers in PCR for each target nucleic acid, including a set for hCMV DNA. By "matched" primers is meant primers having melting temperatures (T_m's) which are essentially the same, that is, they differ by no more than about 5°C. Moreover, the T_m's of the two primers of each set are within the range of from about 65 to about 74°C, and the two primers have nucleotide lengths which

differ from each other by no more than 5 nucleotides.

Further, all of the primers of all primer sets used in the amplification method are also "matched", that is, they all have T_m's which differ by no more than about 5°C and all are within the range of from about 65 to about 74°C.

As one can see from the appealed claims (Appendix A), each primer of each primer set is present in the composition, test kit or method in an amount of from about 0.1 to about 2 μmolar, and the thermostable DNA polymerase is used as relatively high levels, i.e. at least 10 units/100 μl. These parameters enable rapid cycling in the PCR process. That is, the cycle of priming, primer extension product formation and denaturation can proceed most rapidly (on the order of 2 minutes or less per cycle). As will be pointed out below, this is a valuable achievement in view of what was known in the art.

All of these features then are required to achieve rapid, efficient and simultaneous amplification and detection of mutiple targets, one of which is hCMV DNA.

IV. ISSUES

There are two issues in this appeal:

(1) Whether the claims are definite in view of Appellants' teaching in the specification and what it known in the art; and

(2) Whether the claims are patentable over the teaching of the cited art.

Appellants respectfully submit that each issue should be answered in the affirmative, and therefore in Appellants' favor.

V. GROUPING OF CLAIMS

Claims 1-8, 10-13, 16-20, 22-27, 30, 31, 33 and 35-37 are to be considered in one group, and Claims 39-42

are to be considered a second group for consideration, with respect to both issues.

VI. ARGUMENTS

A. Introduction

The Final Rejection has objected to the functional definition of oligonucleotides in Appellants' claims, and rejected them under Section 112(2). Appellants will present arguments that this rejection is clearly erroneous.

In addition, Appellants present arguments below which are supportive of their position that the Final Rejection fails to present a *prima facie* case for obviousness for all claims, but particularly for Claims 39-42 which are considerably more specific than the other claims on appeal.

The Board's consideration of these arguments and agreement with Appellants' positions are respectfully requested.

B. The Rejection Under Section 112(2) Is In Error Because the Claim Language Is Expressed in a Proper Functional Manner

Claims 1-8 and 10-38 have been finally rejected as being indefinite for failing to recite specific oligonucleotide sequences.

The Final Rejection is considered to be seeking to limit all claims to specific oligonucleotide sequences when the Statute does not require it. Clearly, generic compounds have been patented in the past in various technologies using functional limitations and features without the need for specific chemical compositional formulae. Clearly, Appellants intend to claim every sequence that falls within the claim parameters because such relationships have not been described in the art for amplification compositions, kits and methods. Appellants clearly indicate in their specification how such primers can be chosen and used, and teach representative primers in such a manner that the metes and bounds of the claims

are understandable to those skilled in the art, which even the Final Rejection alleges, are doctoral scientists. Thus, those reading this application in the field of endeavor are quite bright and have considerable training in this field. Clearly, such skilled people can understand from Appellants' claims and specification how to choose matched primers for use in the claimed invention for any given target nucleic acid sequence.

Appellants are claiming aqueous solutions, test kits and methods of using certain "matched" primers in PCR. While more specific structural claiming may be called for in "composition of matter" claims, Appellants' claims do not fall into that category.

Applicants believed that they are allowed by the Statute to recite the use of chemical compounds (albeit biological chemical compounds) in functional terms, i.e. by what the primers and probes do, not by their structure, *In re Fuetterer* 138 U.S.P.Q. 217 (C.C.P.A., 1963). The metes and bounds of Appellants claimed invention are well within the routine experimentation of a skilled artisan because Appellants have provided sufficient teaching in their specification as to how to choose appropriate primers and probes (pages 11-16). Other knowledge in the art from the rapidly expanding literature relating to PCR would readily be available also to help one to adequately choose the appropriate sequences to fall within the scope of the claims. Conversely, a skilled artisan would know how to design around the invention because the metes and bounds are clearly defined in the claim language and specification.

As the Board understands, the claims must not be read in a vacuum, but in light of the specification and what is well known in the art, and thereby given the broadest interpretation possible, *In re Marosi et al* 218 U.S.P.Q. 289, at 292 (C.A.F.C., 1983). Because one following this law would understand Appellants' functional definition of primers, the objection to the claims is in error.

These arguments notwithstanding, Appellants' claims 39-42 submitted after the Final Rejection, should not be subject to the same rejection since they recite specific oligonucleotides for the first and second primers and/or first capture probes. Thus, the concerns in the Final Rejection about claiming every possible sequence is not relevant to those claims. Specific oligonucleotides for the third and fourth primers and/or second capture probe are not recited, however, because they are not as critical to the practice of the invention as are the other reagents, as long as all reagents have the properties recited in the claims (i.e., T_m, etc.).

Even if the Board agrees with the Final Rejection with respect to the broader claims, it is respectfully requested that the Final Rejection be reversed with respect to Claims 39-42.

C. Description of Cited Art

1. Nedjar et al Publication

Nedjar et al relates to coamplification of HCV (hepatitis C virus) DNA and HIV-I DNA. It does not relate to detection of hCMV DNA. It is clear from a careful reading of Nedjar et al that it teaches the use of "nested" PCR (see page 299) for detection of two target nucleic acids. As one skilled in the art understands, "nested" PCR requires the use of different primer sets in sequence, not simultaneously.

2. Brytting et al Publication

Brytting relates to amplification of hCMV DNA as opposed to HCV, but it also teaches "nested" PCR (see pages 129-131) using nested primer pairs. Moreover, different temperatures were used in amplification for primer annealing and primer extension (page 131, first paragraph). Moreover, Brytting et al teaches the use of only 1 unit DNA polymerase/50 µl of solution (or 2 units/100 µl).

3. Gibbs et al Publication

Gibbs et al is cited for its alleged motivation to simultaneously detect several target nucleic acids using multiple primer sets. This

publication is a detailed analysis of research for finding optimum primer sets for various sequences in the hypoxanthine phosphoribosyltransferase gene. The reference suggests that the desired primers have a critical amount of GC content.

Gibbs et al describes a process for finding out the optimum amounts of primers needed for detecting multiple sequences on the same gene (see paragraph bridging pages 236-237). Because the multiple targets were detected using gels, they were necessarily of different lengths, and thus different amounts of primers were also necessary. Gibbs et al also teaches that the amounts of each primer set had to be adjusted to compensate for uneven signal strength when they were used in the same reaction mixture (see page 238, paragraph bridging the columns). The amounts of primers ranged from 10 pmol to 25 pmol.

In addition, Gibbs et al teaches multiple amplification using only 8 units DNA polymerase/100 µl, and separate temperatures for primer annealing and primer extension (see paragraph bridging pages 236-7). Each cycle lasted at least 3 minutes. Detection of the multiple analytes in Gibbs et al was achieved by using conventional gels, not "matched" capture probes as in the present invention.

4. WO 90/08840 (Findlay et al)

Findlay et al teaches various diagnostic elements or articles having capture probes disposed thereon.

D. No *Prima Facie* Case for Obviousness Has Been Made in the Final Rejection Especially in View of the Amendments to Appellants' Claims

The claims on appeal recite a limited amount of each primer and a relatively high amount of DNA polymerase, which amounts Appellants submit are important for rapid, efficient and simultaneous amplification in their multiplexing invention. Thus, while the matching Tm's are important, as stated in the specification, the

amount of primer and DNA polymerase are also very important in order to achieve the desired simultaneous amplification in a rapid fashion (each cycle being less than 120 seconds).

Moreover, method Claim 22 recites that each amplification cycle is carried out using the same temperature for primer annealing and primer extension, which temperature is in a narrow range to suit the matched primers used in the method. Thus, that claim more clearly distinguishes the method from routine PCR where one temperature is used for primer annealing and another temperature is used for primer extension. Appellants have found that certain sets of primers, when "matched" as recited in the claims, can be advantageously used to amplify multiple targets simultaneously, as opposed to sequentially, in the presence of specific amounts of primers and DNA polymerase. Appellants' so-called "two-temperature" PCR (one temperature for denaturation, and the second for primer annealing and extension) requires the "matched" primers for multiple target amplification, and such a method is not suggested by the combined art cited in the Final Rejection which teaches "three-temperature" PCR (separate temperatures for denaturation, primer annealing and primer extension).

The rapid cycle (less than 120 seconds) for Appellants' method is highly desirable, and achievable for multiple targets only with the use of the recited amounts of primer and DNA polymerase and the use of "matched" primers which are thereby very efficient under the stringent PCR conditions required for rapidity and efficiency. None of the cited art, alone or in combination, suggests this critical combination of features needed for rapid and efficient amplification of multiple target nucleic acids.

Claims 39-42 are more clearly distinguishable over the cited art because they recite specific oligonucleotides for the first and second primers and first capture probe which are not taught or suggested by the cited art. Thus, even if the Final Rejection is

upheld for the original claims, it should be reversed with respect to Claims 39-42.

Upon review of Nedjar et al, it can be seen that it describes coamplification of HCV and HIV-I DNA, albeit using a "nested" PCR format. However, it does not relate to detection of hCMV DNA. Thus, the characterization of the Final Rejection on page 6 that the "DNA sequence of human CMV is taught in the prior art which has been used by both Nedjar et al and Brytting et al to design primers..." is incorrect.

In the first round of the PCR process, primers for HIV-I and HCV DNA are in the reaction mixture. The T_m values for the HIV-I DNA primers are 66 and 65.5°C, and the values for the HCV DNA are 66.5 and 63.1°C. Thus, even if this reference could conceivably teach the coamplification of hCMV DNA and another target, the four primers taught in Nedjar et al do not fall within Appellants' claims. They do not all have the requisite T_m values required in Appellants' invention.

Moreover, Nedjar et al teaches a very slow PCR procedure (at least 8 minutes per cycle, see page 299) compared to Appellants' very rapid (two minutes or less per cycle) method. Further, Nedjar et al teaches two different primer annealing and extension temperatures (37°C and 72°C) which necessitate very long cycle times since lengthy times are needed for moving from one temperature to another ("ramping" times). Appellants' claims recite a single temperature for both steps. Still further, Nedjar et al teaches the use of merely 2.5 units of DNA polymerase per 100 µl of solution. This is contrasted with Appellants' claims reciting at least 10 units/100 µl.

Clearly then, Nedjar et al is severely deficient as a primary reference in teaching the presently claimed invention. The question is whether the secondary references provide the missing teaching or any motivation to put the missing pieces together. Appellants submit that they do not.

Brytting et al fails to provide any teaching to overcome the deficiencies of Nedjar et al. Admittedly, it relates to amplification of hCMV DNA as opposed to HCV, but it also teaches "nested" PCR (see pages 129-131) using nested primer pairs. Moreover, different temperatures were used in amplification for primer annealing and primer extension (page 131, first paragraph). Contrary to that teaching, Appellants claim PCR using multiple primer sets simultaneously ("non-nested" PCR) and a single temperature for primer annealing and extension.

Moreover, Brytting et al teaches the use of only 1 unit DNA polymerase/50 µl of solution (2 units/100 µl). Admittedly, the Brytting et al cycle was shorter than most (90 seconds), but there is no suggestion that multiple targets can be amplified this quickly in the same reaction mixture. Rather, the "fast" cycles are used in "nested" PCR where primer sets are used in different cycles. Appellants' invention using "matched" primers is not suggested by Brytting et al and Nedjar et al together because the critical features of Appellants' claims are still missing from that combined teaching.

Findlay et al admittedly teaches various diagnostic elements or articles having capture probes disposed thereon. However, it fails to overcome the deficiencies noted above in Brytting et al and Nedjar et al.

It should be noted also that the existence of Findlay et al is supportive of Appellants' argument that the Section 112(2) rejection of the element claim is in error since the basic structure of such articles is well known.

Applicants, however, have provided a novel and patentable improvement by putting "matched" probes on such articles for use in the rapid PCR method recited in Claim 22.

Gibbs et al is cited for its alleged motivation to simultaneously detect several target nucleic acids using multiple primer sets. This is admittedly more

pertinent art that any of Nedjar et al, Brytting et al and Findlay et al. However, it fails to teach or suggest Appellants' claimed invention, by failing to provide the teaching missing from the other cited publications.

Appellants' claimed method is distinguishable thereover. Their method is a "rapid" PCR procedure for amplification of multiple targets wherein each cycle is 120 seconds or less. In order to accomplish this, Appellants require "matched" primers, the same concentration of each primer and a high amount of DNA polymerase.

Gibbs et al is a detailed research study for finding optimum primer sets for various sequences in the hypoxanthine phosphoribosyltransferase gene (see paragraph bridging pages 236-237). The reference suggests that the desired primers have a critical amount of GC content. Because the multiple targets were detected using gels, they were necessarily of different lengths, and thus different amounts of primers were also necessary. Gibbs et al teaches that the amounts of each primer set had to be adjusted to compensate for uneven signal strength when they are used in the same reaction mixture (see page 238, paragraph bridging the columns). The amounts of primers ranged from 10 pmol to 25 pmol which is several orders of magnitude less than Appellants' range of from about 0.1 to about 2 μmolar.

In addition, Gibbs et al teaches multiple amplification using only 8 units DNA polymerase/100 μl, and separate temperatures for primer annealing and primer extension (see paragraph bridging pages 236-7). Each cycle required at least 3 minutes. Detection of the multiple analytes in Gibbs et al was achieved by using conventional gels, not "matched" capture probes as in the present invention.

Thus, Gibbs et al does not suggest the use of considerably more of each primer, rapid cycles and use of the same temperature for primer annealing and primer extension as called for in the present invention. These combined features enable rapid and efficient

amplification and detection of multiple targets by means of multiple capture probes, in contrast to Gibbs et al.

Gibbs et al merely suggests that one can detect multiple targets if one optimizes the amount of each primer used and if the targets are of different lengths. Appellants' method is not under such constraints. The amount of primer need not be optimized within the recited range, and the targets can be of any length because capture is not dependent upon resolution on gels. Moreover, Appellants have a more rapid process and have avoided the need for multiple temperatures in each PCR cycle. Only Appellants' combination of "matched" primers, and primer and DNA polymerase amounts make this possible.

The Final Rejection has failed to present a *prima facie* case for obviousness. While some pieces of the claimed invention are shown in the art, the combination of critical features is lacking in actual description and suggestion. No nexus between the cited art and the claimed invention has been pointed out, and it is merely an opinion that one skilled in the art would be able, with reasonable predictability, achieve what Appellants have done, particularly in view of the amendments to the claims. Thus, the Final Rejection should be reversed.

VII. CONCLUSION

Appellants have presented arguments that the Final Rejection of the claims under both Sections 112(2) and 103 are incorrect. The claim language is definite because the functional definition of the primers is suitably provided in Appellants' specification. Such definition would enable the highly skilled artisan to know the metes and bounds of the claimed invention.

In addition, Appellants' claimed invention is patentable over the prior art because no *prima facie* case for obvviousness has been made.

In the event that the Board agrees with the Final Rejection on any ground with respect to the broader claims, Appellants respectfully submit that Claims 39-42 should be allowed as they are very narrow in scope and avoid all of the objections stated in the Final Rejection.

Early action by the Board to address these issues is respectfully requested.

Respectfully submitted,

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APPENDIX A: CLAIMS ON APPEAL

1. An aqueous composition buffered to a pH of from about 7 to about 9, which comprises:

(a) first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of human cytomegaloviral DNA (hCMV DNA) and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

(b) third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 μmolar, and

(c) a thermostable DNA polymerase present at at least 10 units/100 μl.

2. The composition of claim 1 wherein said composition further comprises a DNA polymerase cofactor present at from about 2 to about 15 mmolar, and

a dNTP present at from about 0.25 to about 3.5 mmolar.

3. The composition of claim 1 wherein each of said first, second, third and fourth primers has a T_m

within the range of from about 67 to about 74°C, all of said primer T_m's being within about 2°C of each other.

4. The composition of claim 1 wherein said first and second primers have nucleotide lengths in the range of from 20 to 35, which lengths differ from each other by no more than 2 nucleotides.

5. The composition of claim 1 wherein said first and second primers have the same length in the range of from 20 to 30 nucleotides.

6. The composition of claim 1 wherein said T_m values are calculated using the formula:

$$T_m\ (°C) = 67.5 + 0.34(\%\ G + C) - 395/N$$

wherein G and C represent the number of guanine and cytosine nucleotides, respectively, and N represents the total number of nucleotides.

7. The composition of claim 6 wherein said third and fourth primers are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a DNA different from hCMV DNA and which is associated with an infectious agent.

8. The composition of claim 7 wherein said third and fourth primers are specific to and hybridizable with said third and fourth nucleic acid sequences which are in opposing strands of a DNA selected from the group consisting of a retroviral DNA, *Mycobacterium tuberculosis* DNA, *Mycobacterium avium* DNA, Epstein Barr viral DNA, respiratory syncytial viral DNA, *Pneumocystis carinii* DNA and hepatitis DNA.

10. The composition of claim 1 wherein one or both of said first and second primers, and one or both of said third and fourth primers, are labeled with the same or different specific binding moiety.

11. The composition of claim 10 wherein said labeled primers are labeled with biotin.

12. A diagnostic test kit for the amplification of human cytomegaloviral DNA and a second target DNA comprising, in separate packaging:

a) an aqueous composition buffered to a pH of from about 7 to about 9, which comprises:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of human cytomegaloviral DNA (hCMV DNA) and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 μmolar, and

a thermostable DNA polymerase present at at least 10 units/100 μl,

b) at least one additional PCR reagent, and

c) a capture reagent comprising a water-insoluble support to which is covalently attached a capture probe which is specific to a nucleic acid sequence of a strand of hCMV DNA, said capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

a second capture reagent comprising a water-insoluble support to which is covalently attached a second capture probe which is specific to a nucleic acid sequence of a strand of said second target DNA, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and being hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C.

13. The test kit of claim 12 wherein said additional PCR reagent is a thermostable DNA polymerase, a DNA polymerase cofactor or a dNTP.

16. The test kit of claim 12 wherein said composition further comprises a DNA polymerase cofactor present at from about 2 to about 15 mmolar, and

a dNTP present at from about 0.25 to about 3.5 mmolar.

17. The test kit of claim 12 wherein one or both of said first and second primers, and one or both of said third and fourth primers are labeled with biotin, and said test kit further includes a conjugate of avidin with an enzyme and a substrate reagent which provides a detectable signal in the presence of said enzyme.

18. The test kit of claim 17 wherein said conjugate comprises avidin and peroxidase, and said substrate reagent provides a detectable colorimetric or chemiluminescent signal in the presence of peroxidase and an oxidant.

19. A diagnostic test kit for the amplification of human cytomegaloviral DNA and a second target DNA comprising, in separate packaging:

a) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of hCMV DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

each of said first and second primers being present in the same amount within the range of from about 0.1 to about 2 μmolar and having a T_m within the range of from about 65 to about 74°C, said primer T_m's being within about 5°C of each other, and

said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides,

b) a separate aqueous composition buffered to a pH of from about 7 to about 9, and comprising third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA and which are separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides,

each of said third and fourth primers being present in the same amount of from about 0.1 to about 2 μmolar and having a T_m within the range of from about 65 to about 74°C, said third and fourth primer T_m's being within about 5°C of each other and within about 5°C of the T_m's of said first and second primers, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, [and]

c) included in either a) or b), a thermostable DNA polymerase present at at least 10 units/100 μl,

d) at least one additional PCR reagent, and

e) a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of a strand of hCMV DNA, said first capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

a second capture reagent comprising a water-insoluble support to which is covalently attached a

second capture probe which is specific to a nucleic acid sequence of a strand of said second target DNA, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

said first and second capture probes having T_m's which differ by no more than about 15°C.

20. The test kit of claim 19 wherein said third and fourth primers are specific to and hybridizable with said third and fourth nucleic acid sequences which are in opposing strands of a DNA selected from the group consisting of a retroviral DNA, *Mycobacterium tuberculosis* DNA, *Mycobacterium avium* DNA, Epstein Barr viral DNA, respiratory syncytial viral DNA, *Pneumocystis carinii* DNA and hepatitis DNA.

22. A method for the amplification and detection of human cytomegaloviral DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of hCMV DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of hCMV DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 μmolar, and

ii) the following additional PCR reagents: a thermostable DNA polymerase present in an amount of at least 10 units/100 μl, a DNA polymerase cofactor and at least one dNTP, any or all of said additional PCR reagents being in the same or a different composition as defined in i),

to simultaneously amplify said opposing hCMV DNA strands and the opposing second target DNA strands wherein, in each PCR cycle, priming and primer extension are carried out at the same temperature within the range of from about 62 to about 75°C and carried out within 120 seconds,

B) capturing one of said amplified hCMV DNA strands with a capture reagent comprising a water-insoluble support to which is covalently attached a capture probe which is specific to a nucleic acid sequence of said hCMV DNA strand, said capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

capturing one of said amplified second target DNA strands with a second capture reagent comprising a second capture probe specific to a nucleic acid sequence of said second target DNA strand, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic

acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

said first and second capture probes having T_m's which differ by no more than about 15°C, and

C) simultaneously detecting said captured amplified hCMV DNA strand and said captured amplified second target DNA strands as a simultaneous determination of the presence of hCMV DNA and said second target DNA.

23. The method of claim 22 wherein each of said first, second, third and fourth primers has a T_m within the range of from about 67 to about 74°C, said primer T_m's being within about 2°C of each other.

24. The method of claim 22 wherein one or both of said first and second primers, and one or both of said third and fourth primers, are labeled with a specific binding moiety.

25. The method of claim 24 wherein said labeled primers are labeled with biotin, and detection of the resulting biotinylated amplified DNA strands for either target DNA is achieved by reacting said biotinylated amplified hCMV DNA strand with an avidin-enzyme conjugate, followed by reaction of said enzyme with a substrate reagent to produce a detectable colorimetric or chemiluminescent signal.

26. The method of claim 25 wherein said biotinylated amplified DNA strands are detected by contacting them with an avidin-peroxidase conjugate, followed by reaction of peroxidase, in the presence of an oxidant, with either: luminol to produce a detectable chemiluminescent signal, or a leuco dye to produce a detectable colorimetric signal.

27. The method of claim 22 wherein PCR is carried out for from 20 to 50 cycles.

30. The method of claim 22 wherein said water-insoluble support for each capture reagent is a polymeric or magnetic particle having a diameter in the range of from about 0.001 to 10 μmeters, and each of said capture probes has a T_m greater than about 55°C.

31. The method of claim 22 wherein said first and second capture reagents are disposed in distinct regions on a water-insoluble substrate of a test device.

33. The method of claim 22 wherein said second target DNA is selected from the group consisting of a retroviral DNA, *Mycobacterium tuberculosis* DNA, *Mycobacterium avium* DNA, Epstein Barr viral DNA, respiratory syncytial viral DNA, *Pneumocystis carinii* DNA and hepatitis DNA.

35. The method of claim 22 wherein a DNA polymerase cofactor is present at from about 2 to about 15 mmolar, and

a dNTP is present at from about 0.25 to about 3.5 mmolar.

36. The method of claim 22 wherein a third target DNA is amplified and detected simultaneously with the hCMV DNA and said second target DNA,

said third target DNA being amplified using a third set of primers wherein each primer has a T_m within the range of from about 65 to about 74°C, the primer T_m's being within 5°C of each other and within 5°C of said first, second, third and fourth primers, and the lengths of primers in said third primer set differing by no more than 5 nucleotides.

37. A diagnostic element comprising a water-insoluble, heat or ultrasonic sealable support, having disposed thereon in distinct regions thereof, a plurality of capture reagents,

each capture reagent having a capture probe specific for and hybridizable with a distinct target DNA at a temperature of from about 40 to about 55°C, each of said capture probes having from 10 to 40 nucleotides and a T_m greater than about 50°C, and the T_m's of all capture probes differing by no more than about 15°C,

at least one of said capture probes being specific for and hybridizable with hCMV DNA.

39. An aqueous composition buffered to a pH of from about 7 to about 9, which comprises:

(a) first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of human cytomegaloviral DNA (hCMV DNA) and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

(b) third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 μmolar, and

said first and second primers being SEQ ID NO:1 and SEQ ID NO:2, respectively, in Primer set 1, or said first and second primers being SEQ ID NO:3 and SEQ ID NO:4, respectively, in Primer set 2, identified as follows:

Primer set 1:

SEQ ID NO:1 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

SEQ ID NO:2 5'-CAGCACCATC CTCCTCTTCC TCTGG-3',

and

Primer set 2:

SEQ ID NO:3 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

SEQ ID NO:4 5'-CACCACGCAG CGGCCCTTGA TGTTT-3', and

(c) a thermostable DNA polymerase present at at least 10 units/100 µl.

40. A diagnostic test kit for the amplification of human cytomegaloviral DNA and a second target DNA comprising, in separate packaging:

a) an aqueous composition buffered to a pH of from about 7 to about 9, which comprises:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of human cytomegaloviral DNA (hCMV DNA) and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 µmolar, and

said first and second primers being SEQ ID NO:1 and SEQ ID NO:2, respectively, in Primer set 1, or said first and second primers being SEQ ID NO:3 and SEQ ID NO:4, respectively, in Primer set 2, identified as follows:

Primer set 1:

SEQ ID NO:1 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

SEQ ID NO:2 5'-CAGCACCATC CTCCTCTTCC TCTGG-3',

and

Primer set 2:

SEQ ID NO:3 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

SEQ ID NO:4 5'-CACCACGCAG CGGCCCTTGA TGTTT-3', and

a thermostable DNA polymerase present at at least 10 units/100 μl,

b) at least one additional PCR reagent, and

c) a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of a strand of hCMV DNA, said capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

a second capture reagent comprising a water-insoluble support to which is covalently attached a second capture probe which is specific to a nucleic acid sequence of a strand of said second target DNA, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and being hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

wherein said first capture probe is selected from the group consisting of:

SEQ ID NO:5 5'-GGTGTCACCC CCAGAGTCCC CTGTACCCGC-3',

SEQ ID NO:6 5'-GACACAGTGT CCTCCCGCTC CTCCTGAGCA-3',

SEQ ID NO:7 5'-GTGGAAGGCG GCTCGCTGGA AGCCGGTCGT-3',

and

SEQ ID NO:8 5'-GAACCGAGGG CCGGCTCACC TCTATGTTGG-3'.

41. A diagnostic test kit for the amplification of human cytomegaloviral DNA and a second target DNA comprising, in separate packaging:

a) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising first and second primers which are specific to and hybridizable with,

respectively, first and second nucleic acid sequences which are in opposing strands of hCMV DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

each of said first and second primers being present in the same amount within the range of from about 0.1 to about 2 μmolar and having a T_m within the range of from about 65 to about 74°C, said primer T_m's being within about 5°C of each other, [and]

said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

said first and second primers being SEQ ID NO:1 and SEQ ID NO:2, respectively, in Primer set 1, or said first and second primers being SEQ ID NO:3 and SEQ ID NO:4, respectively, in Primer set 2, identified as follows:

Primer set 1:

SEQ ID NO:1 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

SEQ ID NO:2 5'-CAGCACCATC CTCCTCTTCC TCTGG-3',

and

Primer set 2:

SEQ ID NO:3 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

SEQ ID NO:4 5'-CACCACGCAG CGGCCCTTGA TGTTT-3',

b) a separate aqueous composition buffered to a pH of from about 7 to about 9, and comprising third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA and which are separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides,

each of said third and fourth primers being present in the same amount of from about 0.1 to about 2 μmolar and having a T_m within the range of from about 65 to about 74°C, said third and fourth primer T_m's being within about 5°C of each other and within about 5°C of the T_m's of said first and second primers, and said third

and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides,

c) included in either a) or b), a thermostable DNA polymerase present at at least 10 units/100 µl,

d) at least one additional PCR reagent, and

e) a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of a strand of hCMV DNA, said first capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

a second capture reagent comprising a water-insoluble support to which is covalently attached a second capture probe which is specific to a nucleic acid sequence of a strand of said second target DNA, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

said first and second capture probes having T_m's which differ by no more than about 15°C, and

wherein said first capture probe is selected from the group consisting of:

SEQ ID NO:5 5'-GGTGTCACCC CCAGAGTCCC CTGTACCCGC-3',

SEQ ID NO:6 5'-GACACAGTGT CCTCCCGCTC CTCCTGAGCA-3',

SEQ ID NO:7 5'-GTGGAAGGCG GCTCGCTGGA AGCCGGTCGT-3', and

SEQ ID NO:8 5'-GAACCGAGGG CCGGCTCACC TCTATGTTGG-3'.

42. A method for the amplification and detection of human cytomegaloviral DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of hCMV DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of hCMV DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 μmolar, and

said first and second primers being SEQ ID NO:1 and SEQ ID NO:2, respectively, in Primer set 1, or said first and second primers being SEQ ID NO:3 and SEQ ID NO:4, respectively, in Primer set 2, identified as follows:

Primer set 1:

SEQ ID NO:1 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

SEQ ID NO:2 5'-CAGCACCATC CTCCTCTTCC TCTGG-3',

and

Primer set 2:

SEQ ID NO:3 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

SEQ ID NO:4 5'-CACCACGCAG CGGCCCTTGA TGTTT-3', and

ii) the following additional PCR reagents: a thermostable DNA polymerase present in an amount of at least 10 units/100 µl, a DNA polymerase cofactor and at least one dNTP, any or all of said additional PCR reagents being in the same or a different composition as defined in i),

to simultaneously amplify said opposing hCMV DNA strands and the opposing second target DNA strands wherein, in each PCR cycle, priming and primer extension are carried out at the same temperature within the range of from about 62 to about 75°C and carried out within 120 seconds,

B) capturing one of said amplified hCMV DNA strands with a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of said hCMV DNA strand, said capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

capturing one of said amplified second target DNA strands with a second capture reagent comprising a second capture probe specific to a nucleic acid sequence of said second target DNA strand, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

said first and second capture probes having T_m's which differ by no more than about 15°C, and

wherein said first capture probe is selected from the group consisting of:

SEQ ID NO:5 5'-GGTGTCACCC CCAGAGTCCC CTGTACCCGC-3',

SEQ ID NO:6 5'-GACACAGTGT CCTCCCGCTC CTCCTGAGCA-3',

SEQ ID NO:7 5'-GTGGAAGGCG GCTCGCTGGA AGCCGGTCGT-3',

and

SEQ ID NO:8 5'-GAACCGAGGG CCGGCTCACC TCTATGTTGG-3',

and

C) simultaneously detecting said captured amplified hCMV DNA strand and said captured amplified second target DNA strands as a simultaneous determination of the presence of hCMV DNA and said second target DNA.